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Request for grant of a patent

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14SEP02 E748223-2 000524
P01/7700 0.00-0221313.0

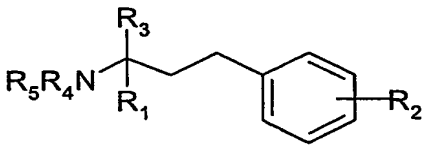
1.	Your reference	4-32619P1	
2.	Patent application number *(The Patent Office will fill in this part)*	0221313.0	
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND	
	Patent ADP number *(if you know it)*	7125487005	
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND	
4.	Title of invention	Organic compounds	
5.	Name of your agent *(If you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Novartis Pharmaceuticals UK Limited Patents and Trademarks Wimblehurst Road Horsham West Sussex RH12 5AB	FS1/77 03.07.03.
	Patents ADP number *(if you know it)*		

6.	If you are declaring priority from one ore more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day/month/year)
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *(see note (d))*	Yes		

Organic Compounds

The present invention relates to amino-propanol derivatives, process for their production, their uses and pharmaceutical compositions containing them.

More particularly, the invention provides a compound of formula I



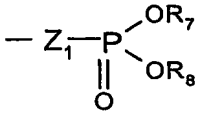
I

wherein

R_1 is C_{1-6} alkyl optionally substituted by OH, C_{1-2} alkoxy or 1 to 6 fluorine atoms; C_{2-6} alkenyl; or C_{2-6}alkynyl;

R_2 is X_1, -O-X_1, -CO-X_1, -CH(OH)-X_1, –C(NOR$_6$)-X_1, -S-X_1, -SO-X_1, -SO_2-X_1 or –N(C_{1-6}alkyl)-X_1 wherein X_1 is C_{3-8} alkyl substituted by 1 to 17 fluorine atoms and optionally interrupted in the carbon chain by one or more O, C=O, CH-OH or C=NOR$_6$ and/or one carbon-carbon double or triple bond; C_{2-8} alkyl-C_{3-6}cycloalkyl wherein the C_{2-8} alkyl moiety is optionally interrupted in the carbon chain by one or more O, C=O, CH-OH or C=NOR$_6$ and/or one carbon-carbon double or triple bond and the C_{3-6}cycloalkyl and/or the C_{2-8} alkyl is substituted by 1 to 17 fluorine atoms; and each of R$_6$, independently, is H, C_{1-4} alkyl, C_{2-4}alkenyl, C_{2-4}alkynyl or benzyl;

R_3 is Z-X_2 wherein Z is CH_2, CHF or CF_2 and X_2 is OH or a residue of formula (a)



(a)

wherein Z_1 is a direct bond, CH_2, CHF, CF_2 or O, and each of R_7 and R_8, independently, is H or C_{1-4} alkyl optionally substituted by 1, 2 or 3 halogen atoms; and

each of R_4 and R_5, independently, is H, C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms, or acyl

in free form or in salt form.

Alkyl or alkyl moiety may be straight or branched chain, e.g. methyl, ethyl, propyl, iso-propyl or butyl. Alkenyl may be e.g. vinyl. Alkynyl may be e.g. propyn-2-yl. Cycloalkyl may be e.g. C_{3-6}cycloalkyl.

Acyl may be a residue R-CO wherein R is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenylC_{1-4}alkyl. Halogen may be F, Cl or Br, preferably F or Cl.

Preferably, the alkyl group or moiety in R_2 contains at least 2 fluorine atoms, more preferably at least 3, particularly from 3 to 8 fluorine carbon atoms. The fluorine atoms preferably replace 1, 2 or 3 hydrogen atoms present on the terminal carbon atoms of the alkyl group or moiety in R_2, i.e. at the ending remote from the phenyl group. By terminal carbon atoms is meant the last, and/or the penultimate, and/or the antepenultimate, etc.. up to the last 8 carbon atoms.

When the cycloalkyl moiety in R_2 is substituted by F, from one up to all hydrogen atoms present in the cycloalkyl moiety may be substituted by F.

R_2 is preferably in position para.

Preferably R_2 is X_1, -O-X_1, -CO-X_1, -CH(OH)-X_1 or $-C(NOR_6)-X_1$, more preferably X_1, -COX_1 or $-O-X_1$.

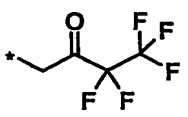
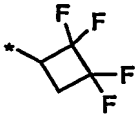
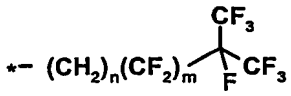
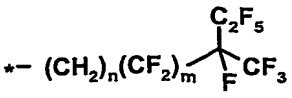
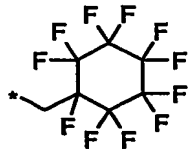
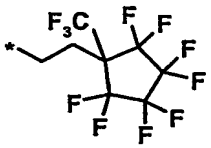
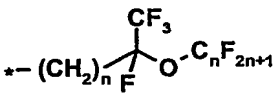
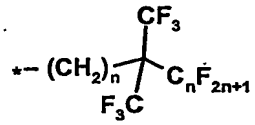
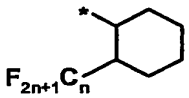
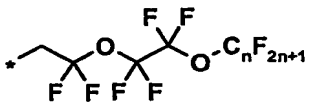
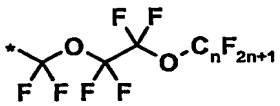
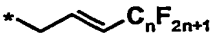
When R_2 does not comprise a cycloalkyl moiety, it is preferably a residue of formula (b)

$$-Y-(CH_2)_n-(CF_2)_m-CH_pF_q \qquad (b)$$

wherein
Y is a direct bond, O, CO, CHOH or $C=NOR_6$ wherein R_6 is as defined above;
n is 0, 1,2, 3, 4 or 5;
m is 0, 1, 2, 3, 4, 5 or 6, provided that the sum of n+m is 3-8
each of p and q, independently, is 0, 1, 2 or 3,
the chain $(CH_2)_n-(CF_2)_m-CH_pF_q$ being optionally interrupted by one carbon-carbon double or triple bond, one CO or one or two oxygen atoms.

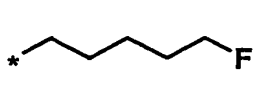
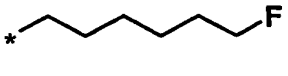
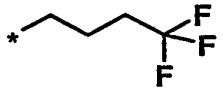
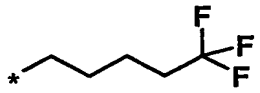
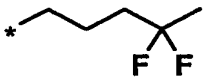
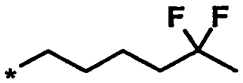
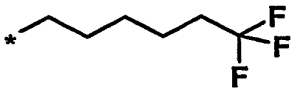
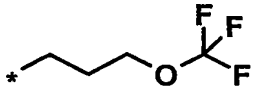
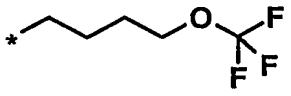
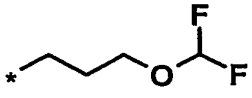
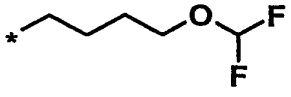
More preferably, R_2 has one of the following significances:
-Y-C_nF_{2n+1} wherein n=3-8 and Y is CH_2, O or C=O;
-Y-$CH_2C_nF_{2n+1}$ wherein n=1-7and Y is CH_2, O or C=O;
-Y-$CH_2CH_2C_nF_{2n+1}$ wherein n=1-6 and Y is CH_2, O or C=O;
-Y-$CH_2CH_2CH_2$ C_nF_{2n+1} wherein n=1-5 and Y is CH_2, O or C=O;
-Y-$(CH_2)_nF$ wherein n=1-7 and Y is CH_2, O or C=O;
-Y-$(CH_2)_nCF_3$ wherein n=1-6 and Y is CH_2, O or C=O;
-Y-$(CH_2)_nCF_2CH_3$ wherein n=1-4 and Y is CH_2, O or C=O;
-Y-$(CH_2)_n(CF_2)_mCHF_2$ wherein n=0-3, m=1-6, n+m = 3-7 and Y is CH_2, O or C=O; or
-Y-$(CH_2)_nC(O)CF_3$ wherein n=1-5 and Y is CH_2, O or C=O.

Further preferred significances for R_2 are e.g.

wherein n and m have one of the significances given above, the sum n+m being 3-8, and the asterisk * means the attachment to the phenyl ring directly or through O, CO, CHOH, $C(NOR_6)$, S, SO, SO_2 or $N(C_{1-6}alkyl)$. Preferably the attachment of R_2 to the phenyl ring is through O.

Further examples of preferred significances for R_2 are e.g.

wherein the asterisk * is as defined above.

Most preferably R_2 is $-O(CH_2)_3CF_2CF_3$, $-O(CH_2)_4CF_2CF_3$, $-O(CH_2)_2CF_2CF_3$, $-(CH_2)_4C_2F_5$, $-(CH_2)_5C_2F_5$, $-(CH_2)_3C_2F_5$, $-C(O)(CH_2)_3CF_2CF_3$, $-C(O)(CH_2)_4CF_2CF_3$ or $-C(O)(CH_2)_2CF_2CF_3$, preferably in position para.

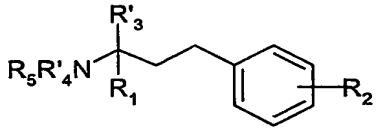
Preferably Z_1 is O.

Compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. inorganic acids, such as hydrochloride, hydrobromide or sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate or benzenesulfonate salts; when R_7 or R_8 is H, the phosphate group may also be present in salt form, e.g. an ammonium salt or salts with metals such as sodium, potassium, calcium, zinc or magnesium, or a mixture thereof. Compounds of formula I and their salts, in hydrate or solvate form are also part of the invention.

When the compounds of formula I have asymetric centers in the molecule, various optical isomers are obtained. The present invention also encompasses enantiomers, racemates, diastereoisomers and mixtures thereof. For example, the central carbon atom bearing R_1, R_3 and NR_4R_5 may have the R or S configuration. Compounds having the R configuration at this central carbon atom are preferred. Moreover, when the compounds of formula I include geometric isomers, the present invention embraces cis-compounds, trans-compounds and mixtures thereof. Similar considerations apply in relation to starting materials exhibiting asymetric carbon atoms or unsaturated bonds as mentioned above, e.g. compounds of formula II, III or IV as indicated below.

The present invention also includes a process for the preparation of a compound of formula I which process comprises

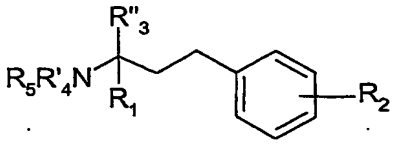
a) for a compound of formula I wherein R_3 is $Z\text{-}X_2$, X_2 being OH, removing the protecting group present in a compound of formula II



II

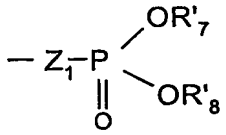
wherein R_1, R_2 and R_5 are as defined above, R'_3 is $Z\text{-}X_2$ wherein X_2 is OH and R'_4 is an amino protecting group, or

b) for a compound of formula I wherein R_3 is $Z\text{-}X_2$, X_2 being a residue of formula (a), removing the protecting groups present in a compound of formula III



III

wherein R_1, R_2, R'_4 and R_5 are as defined above, and R''_3 is $Z\text{-}X_2$ wherein Z is as defined above and X_2 is a residue of formula (a')



(a')

wherein Z_1 is as defined above and each of R'_7 or R'_8 is a hydrolysable or hydrogenolysable group or R'_7 and R'_8 form together a divalent bridging residue optionally fused to a ring (e.g. benzene ring),

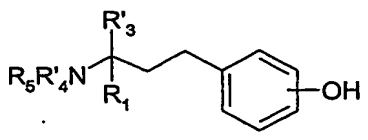
and, where required, converting the compounds of formula I obtained in free form into the desired salt form, or vice versa.

Process step a) may be carried out in accordance with methods known in the art. The removal of the amino protecting groups may conveniently be performed according to methods known in the art, e.g. by hydrolysis, e.g. in an acidic medium, for example using hydrochloric acid. Examples of protecting groups for amino groups are e.g. as disclosed in "Protective Groups in Organic Synthesis" T.W. Greene, J.Wiley & Sons NY, 2nd ed., chapter 7, 1991, and references therein, e.g. benzyl, p-methoxybenzyl, methoxymethyl, tetrahydropyranyl, trialkylsilyl, acyl, tert.-butoxy-carbonyl, benzyloxycarbonyl, 9-fluorenyl methoxy carbonyl, trifluoroacetyl, and the like.

In the residue of formula (a'), each of R'_7 and R'_8 may have the significance of e.g. phenyl or benzyl or form together a cyclic system such as in 1,5-dihydro-2,4,3-benzodioxaphosphepin.

Process step (b) may be performed according to methods known in the art, e.g. by hydrolysis, e.g. in a basic medium when R'_7 and R'_8 are each a hydrolysable group, for example using a hydroxide such as barium hydroxide. It may also be performed by hydrogenolysis, e.g. in the presence of a catalyst, e.g. Pd/C, followed by hydrolysis, e.g. in an acidic medium, for example HCl. Compounds of formulae II and III, used as starting materials, and salts thereof are also novel and form part of the invention.

The present inveniton also includes a process for the preparation of a compound of formula II which process comprises alkylating a compound of formula IV

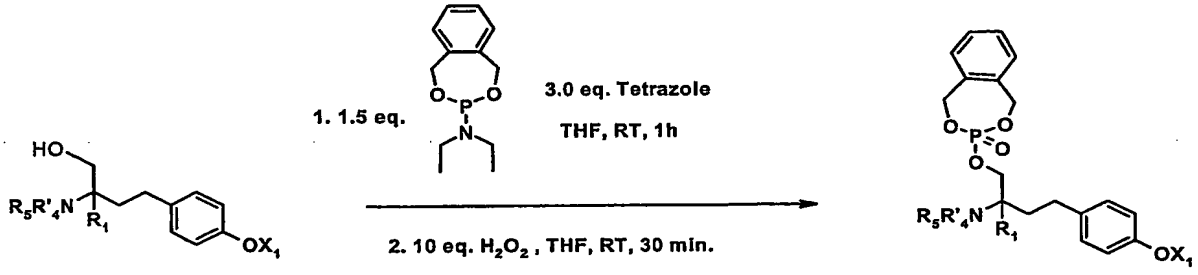


IV

wherein R_1, R'_3, R'_4 and R_5 are as defined above, to introduce the desired residue X_1.

Alkylation of the compounds of formula IV may be performed according to methods known in the art, e.g. by nucleophilic substitution, e.g. by reaction with an alkylating agent X_1-X_3 wherein X_3 is mesylate, tosylate, triflate, nosylate or an halogen, e.g. chloride, bromide or iodide. The alkylation may also be carried out by following the Mitsunobu protocol (e.g. as disclosed in Hughes, Organic Preparations and Procedures International 28, 127-64, 1996 or D.L. Hughes, Org. React. 42, 335, 1992), in solution or on solid phase support synthesis, e.g. by attaching the compound of formula IV to a resin. Alternatively, either triphenylphosphine or e.g. diethyl azocarboxylate bound to a resin, e.g. polystyrene, can be used.

Compounds of formula III wherein R'_7 and R'_8 form a cyclic system, may be prepared as follows:



wherein X_1, R_1, R'_4 and R_5 are as defined above.

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as disclosed in the Examples hereinafter.

The following Examples are illustrative of the invention. Melting points are uncorrected.

RT	=	room temperature
DCM	=	dichloromethane
Bn	=	benzyl
THF	=	tetrahydrofuran
DMF	=	dimethylformamide
MTBE	=	methyl tert.-butyl ether

Example 1: (R)-2-Amino-2-methyl-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-butan-1-ol Hydrochloride

To *tert*-butyl {(R)-1-hydroxy-2-methyl-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-but-2-yl}-carbamate (25 mg, 0.055 mmol) is added 4 M HCl in dry dioxane (1 mL). The clear colorless solution is stirred for 2 h protected from moisture. Then, the solution is evaporated to dryness and the partially crystalline residue is taken up in dry ether (5 mL). Sonication for 10 min gives a precipitate of colorless crystals. The product is filtered off, washed with cold ether (3 x 1 mL), and dried *in vacuo* to afford the title compound in form of a non hygroscopic colorless microcrystalline powder: mp. 186-189°C, ESI+ MS: m/z = 356 (MH^+), ^{1}H-NMR (400 MHz, CD_3OD): δ 1.35 (s, 3H, 2-Me), 1.91 (cm, 2H, 3-CH_2), 2.06 (cm, 2H, 2'-CH_2), 2.35 (cm, 2H, 3'-CH_2), 2.63 (cm, 2H, 4-ArCH_2), 3.55 (d, 1H, ^2J=12.1, 1-CH_α), 3.63 (d, 1H,, ^2J=11.9, 1-CH_β), 4.06 (t, 3H, ^3J=7.1, 1'-OCH_2), 6.88 ('d', 2H, J=11.0, ArH), 7.18 ('d', J=10.8 Hz , ArH).

The required starting material may be prepared according to following procedure:

a) *tert*-Butyl {(R)-1-hydroxy-2-methyl-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-but-2-yl}-carbamate

General Procedure Method A1 (Mitsunobu Reaction using polystyrene-triphenyl phosphine)

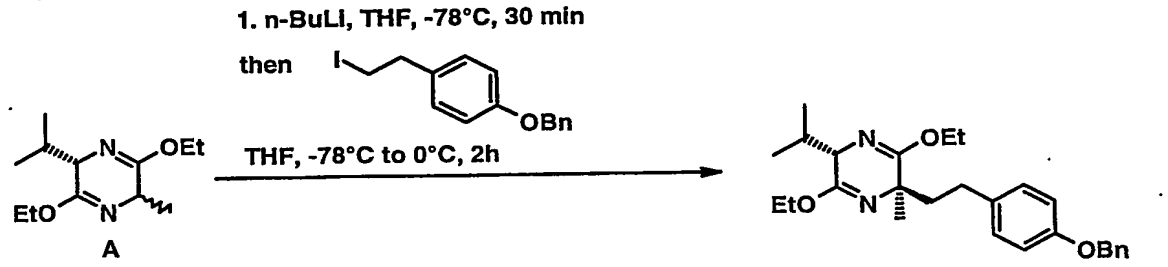
To a solution of *tert*-butyl [(R)-1-hydroxy-4-(4-hydroxy-phenyl)-2-methyl-but-2-yl]-carbamate (100 mg, 0.34 mmol) and 4,4,5,5,5-pentafluoropentan-1-ol (50 µL, 0.37 mmol, 1.1 eq.) in dry THF (5 mL) is added triphenylphosphine-polystyrene 1.10 mmol g^{-1} (370 mg, 0.41 mmol, 1.2 eq.). The suspension is shaken for 15 min to allow the resin to swell. Then, diethyl azodicarboxylate (67 µL, 0.41 mmol, 1.2 eq.) is injected in one portion. The suspension obtained is shaken under argon at RT overnight. Then, the polymer is filtered off and washed with THF (3 x 2 mL). Evaporation of the combined filtrates affords a yellow semi-crystalline residue. Purification by flash chromatography (FlashMaster II, MTBE / hexanes gradient: 0% MTBE -> 30% MTBE within 30 min.; 30% MTBE -> 60% MTBE within 10 min) gives colorless crystals: mp. 90-92°C, ESI+ MS: m/z = 456 (MH^+), 400 (MH^+ - *t*Bu), 356 (MH^+ - Boc), ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.16 (s, 3H, 2-Me), 1.37 (s, 9H, *t*Bu), 1.79 (cm, 1H, 3-CH_α), 1.91-2.04 (m, 3H, 3-CH_α + 2'-CH_2), 2.12-2.28 (m, 2H, 3'-CH_2), 2.51 (cm, 2H, 4-CH_2Ar), 3.58 (d, 1H, ^2J=10.9, 1-CH_α), 3.63 (d, 1H,, ^2J=11.2, 1-CH_β), 3.94 (t, 3H, ^3J=7.3, 1'-ArOCH_2), 6.75 ('d', 2H, J=10.2, ArH), 7.05 ('d', J=10.5, ArH).

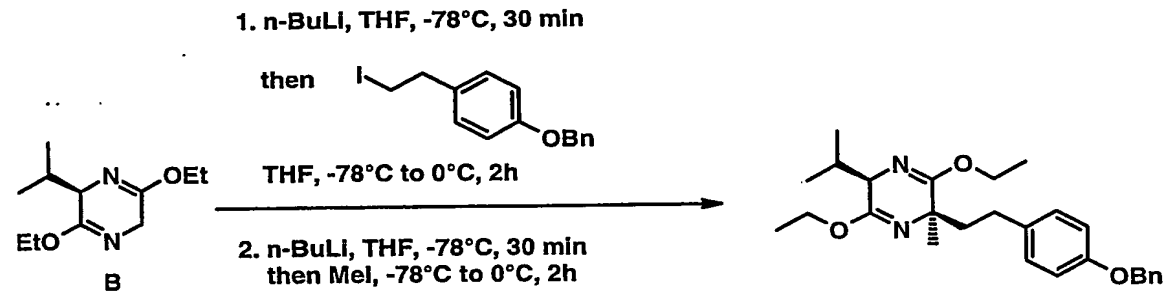
General Procedure Method A2 (Mitsunobu Reaction in solution)

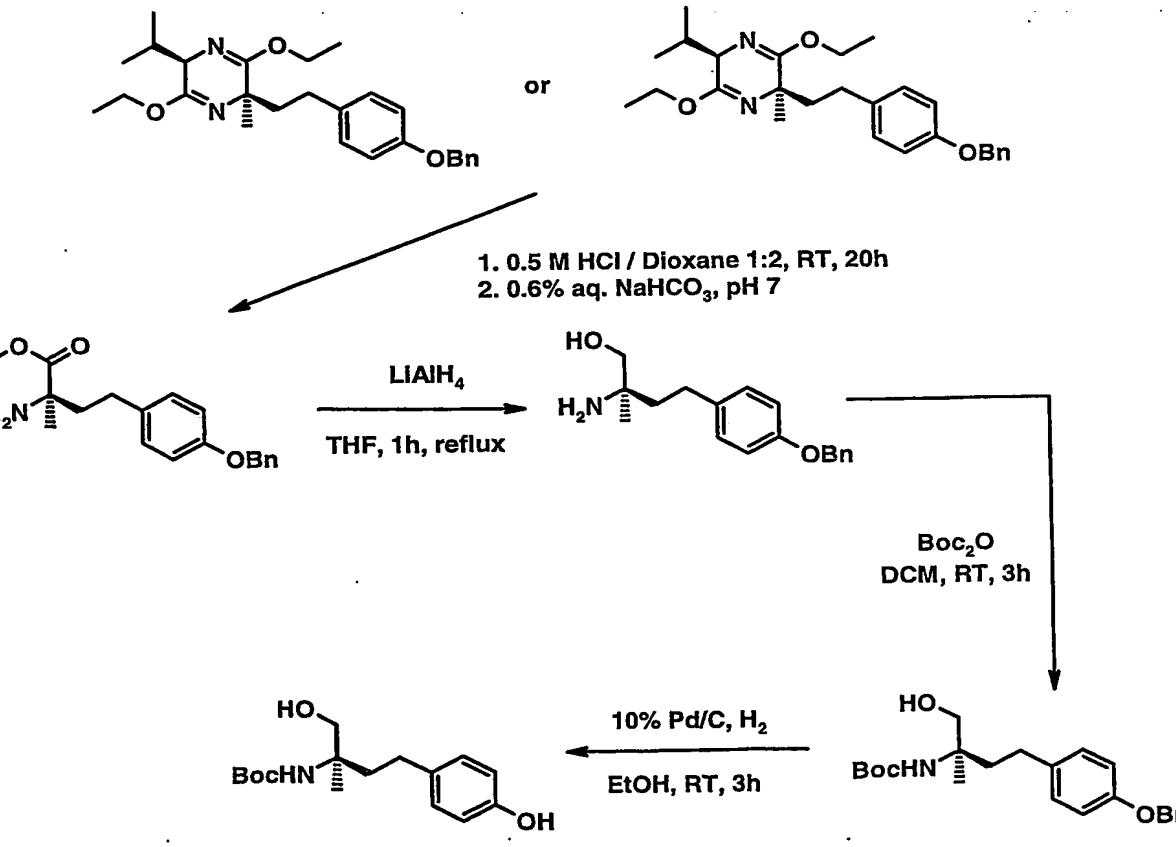
A solution of *tert*-butyl [(R)-1-hydroxy-4-(4-hydroxy-phenyl)-2-methyl-but-2-yl]-carbamate (1.48 g, 5 mmol), 4,4,5,5,5-pentafluoropentan-1-ol (0.74 mL, 5.5 mmol) and triphenyl phosphine (1.39 g, 5.25 mmol) in anhydrous THF (50 mL) is placed in an ice bath. After stirring for 10 min diethyl diazodicarboxylate (0.87 mL, 5.25 mmol) is injected slowly within a period of 15 min. After completion of the addition the ice bath is removed and the now pale yellow reaction mixture is stirred at RT under argon overnight. Then, the solvent is evaporated und the residue recrystallized from MTBE/hexane in order to remove most of the diethyl hydrazinodicarboxylate and triphenyl phosphine oxide formed in the reaction. The mother liquor is evaporated to dryness. Purification by flash chromatography (eluent: MTBE/Hexanes 1:2) affords the title compound as colorless crystals.

b) *tert*-Butyl [(R)-1-hydroxy-4-(4-hydroxy-phenyl)-2-methyl-but-2-yl]-carbamate

The title compound can be prepared according to the scheme depicted below. As starting material Schoellkopf reagents either obtained from L-Valine and Alanine **A** or from D-Valine and Glycine **B** can be used.


1. n-BuLi, THF, -78°C, 30 min
then
I
OBn
THF, -78°C to 0°C, 2h
N
OEt
EtO
N
A
OEt
EtO
N
OBn


1. n-BuLi, THF, -78°C, 30 min
then
I
OBn
THF, -78°C to 0°C, 2h
N
OEt
EtO
N
B
2. n-BuLi, THF, -78°C, 30 min
then MeI, -78°C to 0°C, 2h
N
O
O
N
OBn


N
O
O
N
OBn
or
N
O
O
N
OBn
1. 0.5 M HCl / Dioxane 1:2, RT, 20h
2. 0.6% aq. NaHCO3, pH 7
O
O
H2N
OBn
LiAlH4
THF, 1h, reflux
HO
H2N
OBn
Boc2O
DCM, RT, 3h
HO
BocHN
OBn
10% Pd/C, H2
EtOH, RT, 3h
HO
BocHN
OH

Example 2: (R)-2-Amino-2-methyl-4-[4-(4-methyl-pentyloxy)-phenyl]-butan-1-ol Hydrochloride

Deprotection of *tert*-butyl {(R)-1-Hydroxy-2-methyl-4-[4-(4-methyl-pentyloxy)-phenyl]-but-2-yl carbamate performed as disclosed in Example 1 affords the title compound as a non hygroscopic colorless powder: mp. 168-171°C, ESI+ MS: m/z = 280 (MH^+), ^{1}H-NMR (400 MHz, CD_3OD): δ 1.15 (d, 6H, *J*=7.7, $CH(C\underline{H}_3)_2$), 1.52 (s, 3H, 2-Me), 1.55 (cm, 2H, 3'-CH_2), 1.82 (cm, 1H, $C\underline{H}(CH_3)_2$), 1.97 (cm, 2H, 2'-CH_2), 2.10 (cm, 2H, 3-CH_2), 2.81 (cm, 2H, 4-$ArCH_2$), 3.73 (d, 1H, ^2J=10.9, 1-CH_α), 3.83 (d, 1H, ^2J=10.8, 1-CH_β), 4.03 (t, 3H, ^3J=6.6, 1'-OCH_2), 7.05 ('d', 2H, *J*=9.9, ArH), 7.34 ('d', *J*=10.1 Hz , ArH).

The required starting material may be prepared according to the following procedure:

a) *tert*-Butyl {(R)-1-hydroxy-2-methyl-4-[4-methylpentyloxy)-phenyl]-but-2-yl}-carbamate

General Procedure Method B (Alkylation Reaction)

To a solution of *tert*-butyl [(R)-1-hydroxy-4-(4-hydroxy-phenyl)-2-methyl-but-2-yl]-carbamate (100 mg, 0.34 mmol, Ex. 1b) and 1-bromo-4-methylpentane (75 µL, 0.51 mmol, 1.5 eq.) in anhydrous DMF (2.5 mL) is added water free caesium carbonate (166 mg, 0.51 mmol, 1.5 eq.). The suspension obtained is stirred over night protected from moisture at 60°C. After cooling to RT the solids are filtered off and rinsed with DMF (2 x 1 mL). The combined filtrates are evaporated in a high vacuum to give a dark orange syrup. Purification by flash chromatography (FlashMaster II, MTBE/hexanes gradient as disclosed in Ex 1a)) gives colorless crystals: mp. 94-96°C, ESI+ MS: m/z = 402 (MNa^+), 380 (MH^+), 324 (MH^+ - *t*Bu), ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.94 (d, 6H, *J*=7.8, $CH(C\underline{H}_3)_2$), 1.24 (s, 3H, 2-Me), 1.35 (cm, 2H, 3'-CH_2), 1.46 (s, 9H, *t*Bu), 1.62 (cm, 1H, $C\underline{H}(CH_3)_2$), 1.80 (cm, 2H, 2'-CH_2), 1.87 (cm, 1H, 3-CH_α), 2.05 (cm, 1H, 3-CH_β), 2.49-2.70 (m, 2H, 4-CH_2Ar), 3.67 (d, 1H, ^2J=11.2, 1-CH_α), 3.72 (d, 1H,, ^2J=10.9, 1-CH_β), 3.93 (t, 3H, ^3J=6.1, 1'-$ArOCH_2$), 6.83 ('d', 2H, *J*=10.1, ArH), 7.11 ('d', *J*=10.3, ArH).

Example 3: *Mono*-(R)-2-Amino-2-methyl-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-but-2-yl phosphate

To a solution of *tert*-butyl {(R)-2-Methyl-2-(3-oxo-1,5-dihydro-3λ^5-benzo[e][1,3,2]dioxaphosphepin-3-yloxy)-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-but-2-yl}-carbamate (32 mg, 0.05 mmol) in methanol is added Pd/C 10% (50 mg). The suspension is purged with nitrogen and then hydrogenated at atmospheric pressure with gentle stirring for 2h. Thereafter, the catalyst is filtered off and the filtrate is evaporated to dryness to give a colorless resin. The residue is re-dissolved in dioxane (0.75 mL) and 4 M HCl in dioxane (0.25 mL) is added. After stirring for 2h the slightly turbid solution is evaporated. The colorless semi-solid residue

is sonicated with dry ether (5 mL) to give a colorless precipitate. The solid is filtered off, washed with dry ether and vacuum dried to afford a colorless powder: mp. 229-231°C, ESI– MS: m/z = 434 ($M-H^-$), ^{1}H-NMR (400 MHz, CD_3OD): δ 1.37 (s, 3H, 2-Me), 1.88 (cm, 1H, 3-CH_α), 1.94-2.09 (m, 3H, 3-CH_α + 2'-CH_2), 2.32 (cm, 2H, 3'-CH_2), 2.64 (cm, 2H, 4-CH_2Ar), 3.90 (dd, 1H, ^2J=10.6, $^3J_{H,P}$=4.5, 1-CH_α), 4.00 (dd, 1H, 1-CH_β), 4.03 (t, 3H, ^3J=6.6 Hz, 1'-$ArOCH_2$), 6.88 ('d', 2H, J=10.1, ArH), 7.16 ('d', J=8.1, ArH).

The required starting material can be prepared according to the following procedure:
a) *tert*-butyl {(R)-2-Methyl-2-(3-oxo-1,5-dihydro-3λ^5-benzo[e][1,3,2]dioxaphos-phepin-3-yloxy)-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-but-2-yl}-carbamate
To a solution of *tert*-butyl {(R)-1-hydroxy-2-methyl-4-[4-(4,4,5,5,5-pentafluoro-pentyloxy)-phenyl]-but-2-yl}-carbamate (40 mg, 0.088 mmol, Ex. 1a) and tetrazole (18 mg, 0.26 mmol, 3 eq., recrystallized from toluene) in dry THF (1 mL) is added 3-diethylamino-1,5-dihydro-benzo[e][1,3,2]dioxaphosphepine (32 µL, 0.13 mmol, 1.5 eq.). The reaction mixture is stirred under argon at RT for 3h. Then, H_2O_2 (30%, 90 µL, 0.88, 10 eq.) is injected at 0°C with vigorous stirring. The reaction mixture is stirred for further 30 min, followed by addition of saturated sodium thiosulfate solution (1 mL). The organic layer is separated and the aqueous phase is extracted with ether (3 x 1 mL). The combined organic extracts are washed with brine, dried over $MgSO_4$, and evaporated to dryness. The crude material is purified by flash chromatography (MTBE / Hx 1:1) to afford colorless crystals: ESI+ MS: m/z = 655 (MNH_4^+), 638 (MH^+), 538 (MH^+-Boc).
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 3H, 2-Me), 1.44 (s, 9H, *t*Bu), 1.80 (cm, 1H, 3-CH_α), 2.02-2.20 (m, 3H, 3-CH_α + 2'-CH_2), 2.27 (cm, 2H, 3'-CH_2), 2.59 ('t', 2H, J=8.6, 4-CH_2Ar), 4.02 (t, 3H, ^3J=5.9, 1'-$ArOCH_2$), 4.17 (dd, 1H, ^2J=9.9, $^3J_{H,P}$=5.4, 1-CH_α), 4.35 (dd, 1H, 1-CH_β), 5.17 (dd, 2H, $ArCH_\alpha O$, ^2J=13.6, $^3J_{H,P}$=15.3), 5.30 (ddd, 2H, $ArCH_\beta O$, ^2J=13.4, $^3J_{H,P}$=16.3, J=4.4), 6.82 ('d', 2H, J=8.9, ArH), 7.16 ('d', J=8.7, ArH), 7.29-7.35 (m, 2H, ArH), 7.37-7.42 (m, 2H, ArH).

The compounds of formula I in free form or in pharmaceutically acceptable salt form, exhibit valuable pharmacological properties, e.g. lymphocyte recirculation modulating properties, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. In vitro

The compounds of formula I have binding affinity to individual human S1P receptors as determined in following assays:

Sphingosine-1-phosphate (S1P) receptor profiling

Agonist activities of compounds are tested on the human S1P receptors EDG-1 ($S1P_1$), EDG-3 ($S1P_3$), EDG-5 ($S1P_2$), EDG-6 ($S1P_4$) and EDG-8 ($S1P_5$). Functional receptor activation is assessed by quantifying compound induced GTP [γ-^{35}S] binding to membrane protein prepared from transfected CHO or RH7777 cells stably expressing the appropriate human S1P receptor. The assay technology used is SPA (scintillation proximity based assay). Briefly, DMSO dissolved compounds are serially diluted and added to SPA- bead (Amersham-Pharmacia) immobilised S1P receptor expressing membrane protein (10-20µg/well) in the presence of 50 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 10 µM GDP, 0.1% fat free BSA and 0.2 nM GTP [γ-^{35}S] (1200 Ci/mmol). After incubation in 96 well microtiterplates at RT for 120 min, unbound GTP [γ-^{35}S] is separated by a centrifugation step. Luminescence of SPA beads triggered by membrane bound GTP [γ-^{35}S] is quantified with a TOPcount plate reader (Packard). EC_{50}s are calculated using standard curve fitting software.

For example, the compound of Ex. 3 has an EC_{50} to S1P1 of 16.1 nM, to S1P2 of >10000 nM, to S1P3 of >10000 nM, to S1P4 of 15 nM and to S1P5 of 0.9 nM.

B. In vivo: Blood Lymphocyte Depletion

A compound of formula I or the vehicle is administered orally by gavage to rats. Tail blood for hematological monitoring is obtained on day –1 to give the baseline individual values, and at 2, 6, 24, 48 and 72 hours after application. In this assay, the compounds of formula I deplete peripheral blood lymphocytes when administered at a dose of 0.03 to 3 mg/kg. For example, Compound of Example 1 depletes peripheral blood lymphocytes by more than 50% 6 hours after administration of a dose of 0.2 mg/kg.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by lymphocytes interactions, e.g. in transplantation, such as acute or chronic rejection of cell, tissue or organ allo- or xenografts or delayed graft function, graft versus host disease, autoimmune diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus, hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, vasculitis, pernicious anemia, Sjoegren syndrome, uveitis, psoriasis, Graves ophthalmopathy, alopecia areata and others, allergic diseases, e.g. allergic asthma, atopic dermatitis, allergic rhinitis/conjunctivitis, allergic contact dermatitis, inflammatory diseases optionally with underlying aberrant reactions, e.g. inflammatory bowel disease, Crohn's disease or ulcerative colitis, intrinsic asthma,

inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, atherosclerosis, osteoarthritis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, cutaneous manifestations of immunologically-mediated disorders, inflammatory eye disease, keratoconjunctivitis, myocarditis or hepatitis, ischemia/reperfusion injury, e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, traumatic shock, cancer, e.g. breast cancer, T cell lymphomas or T cell leukemias, infectious diseases, e.g. toxic shock (e.g. superantigen induced), septic shock, adult respiratory distress syndrome or viral infections, e.g. AIDS, viral hepatitis, chronic bacterial infection, or senile dementia. Examples of cell, tissue or solid organ transplants include e.g. pancreatic islets, stem cells, bone marrow, corneal tissue, neuronal tissue, heart, lung, combined heart-lung, kidney, liver, bowel, pancreas, trachea or oesophagus. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired.

In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 2.5 mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 100 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to 50 mg active ingredient.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent.

The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by lymphocytes, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 or 1.2 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 or 1.2 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 or 1.2 above.

The compounds of formula I may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, other drugs e.g. immunosuppressive or immunomodulating agents or other anti-inflammatory agents, e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders, or a chemotherapeutic agent, e.g a malignant cell anti-proliferative agent. For example, the compounds of formula I may be used in combination with a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578 or AP23573; an ascomycin having immunosuppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40. CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a

chemotherapeutic agent, e.g. paclitaxel, gemcitabine, cisplatinum, doxorubicin or 5-fluorouracil; or an anti-infectious agent.

Where the compounds of formula I are administered in conjunction with other immunosuppressive / immunomodulatory, anti-inflammatory. chemotherapeutic or anti-infectious therapy, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory, chemotherapeutic or anti-infectious compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth. In accordance with the foregoing the present invention provides in a yet further aspect:

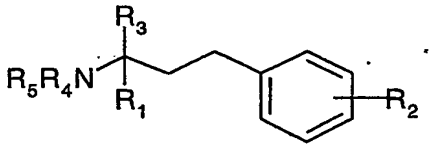
5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a compound of formula I and at least a second drug substance, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a compound of formula I as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory, chemotherapeutic or anti-infectious agent. The kit may comprise instructions for its administration.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.
The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the 2 compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

CLAIMS

1. A compound of formula I



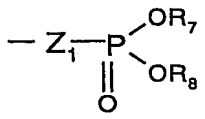
I

wherein

R_1 is C_{1-6} alkyl optionally substituted by OH, C_{1-2} alkoxy or 1 to 6 fluorine atoms; C_{2-6} alkenyl; or C_{2-6}alkynyl;

R_2 is X_1, -O- X_1, -CO- X_1, -CH(OH)- X_1, $-C(NOR_6)-X_1$, -S-X_1, -SO-X_1, $-SO_2-X_1$ or $-N(C_{1-6}alkyl)-X_1$ wherein X_1 is C_{3-8} alkyl substituted by 1 to 17 fluorine atoms and optionally interrupted in the carbon chain by one or more O, C=O, CH-OH or $C=NOR_6$ and/or one carbon-carbon double or triple bond; C_{2-8} alkyl-C_{3-6}cycloalkyl wherein the C_{2-8} alkyl moiety is optionally interrupted in the carbon chain by one or more O, C=O, CH-OH or $C=NOR_6$ and/or one carbon-carbon double or triple bond and the C_{3-6}cycloalkyl and/or the C_{2-8} alkyl is substituted by 1 to 17 fluorine atoms; and each of R_6, independently, is H, C_{1-4} alkyl, C_{2-4}alkenyl, C_{2-4}alkynyl or benzyl;

R_3 is $Z-X_2$ wherein Z is CH_2, CHF or CF_2 and X_2 is OH or a residue of formula (a)



(a)

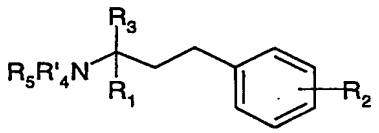
wherein Z_1 is a direct bond, CH_2, CHF, CF_2 or O, and each of R_7 and R_8, independently, is H or C_{1-4} alkyl optionally substituted by 1, 2 or 3 halogen atoms; and each of R_4 and R_5, independently, is H, C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms, or acyl

in free form or in salt form,

a process for its preparation, its use as a pharmaceutical, a pharmaceutical composition containing such a compound or pharmaceutically acceptable salt thereof, a method of treatment or prevention using such a compound or pharmaceutically acceptable salt thereof,

or a pharmaceutical combination comprising such a compound or pharmaceutically acceptable salt thereof, substantially as herein defined and/or described.

2. A compound of formula



wherein R_1 to R_3 and R_5 are as defined in claim 1, and R'_4 is a protecting group, or a salt thereof,

and a process for its preparation, substantially as hereinbefore defined and/or described.